Journal of Medical Virology 50:303–308 (1996)

Superinfection by Homotypic Virus in Hepatitis C Virus Carriers: Studies on Patients With Post-Transfusion Hepatitis

J.H. Kao, P.J. Chen, J.T. Wang, P.M. Yang, M.Y. Lai, T.H. Wang, and D.S. Chen

Department of Internal Medicine (J.H.K., J.T.W., P.M.Y., T.H.W., D.S.C.), Graduate Institute of Clinical Medicine (P.J.C., M.Y.L.), and Hepatitis Research Center (D.S.C.), National Taiwan University Hospital, Taipei, Taiwan

Although heterotypic superinfection and mixed infections of hepatitis C virus (HCV) may be possible for hepatitis flares in chronic hepatitis C, the possibility of homotypic HCV superinfection in HCV carriers with post-transfusion hepatitis has not been explored. Six HCV carriers with post-transfusion non-A, non-B hepatitis found in a prospective study of post-transfusion hepatitis were included. Serum samples before transfusion and during hepatitis were selected to determine genotypes of HCV and nucleotide sequences of the hypervariable region (HVR). The genotypes identified before and after transfusion were concordant in all. There were four with type 1b and one each with type 2a and type 2b. Amplified nucleotide sequences of the HVR before transfusion and during hepatitis were compared in four patients, and a >95% homology was observed in three, suggesting persistence of original viruses. In contrast, only a 51% homogeneity was seen in a given patient, suggesting a homotypic HCV superinfection. Phylogenetic tree analysis validated further these findings. This study implies that HCV carriers can be reinfected by homotypic HCV, and this may contribute to hepatitis flares in chronic hepatitis C. These findings also confirm a weak or inadequate protective immunity in HCV infection and justify protection from reinfection of HCV of patients with chronic hepatitis C. © 1996 Wiley-Liss, Inc.

KEY WORDS: HCV, genotype, homotypic superinfection, mixed infection, RT-PCR, chronic hepatitis C.

INTRODUCTION

Hepatitis C virus (HCV) has been shown to be the major etiologic agent of post-transfusion and sporadic non-A, non-B (NANB) hepatitis worldwide [Alter, 1990]. Patients with acute HCV infection often develop chronic hepatitis, and a significant proportion of these patients develop cirrhosis and eventually hepatocelluar carcinoma [Alter, 1990; Chen, 1994; Kao et al., 1994c; Kiyosawa et al., 1990]. HCV, like many RNA viruses, exhibits genetic heterogeneity [Bukh et al., 1995], and significant sequence diversity has been reported among isolates from different areas [Chen et al., 1992; Houghton et al., 1991; Okamoto et al., 1991] and even within the same individual [Higashi et al., 1993; Martell et al., 1992]. Recent phylogenetic analysis of the nonstructural region 5 of HCV genome has classified the virus into six major groups and 11 subtypes [Simmonds et al., 1993]. Reinfection with heterotypic and homotypic HCV strains has been documented in cross-challenge studies in chimpanzees [Farci et al., 1992; Okamoto et al., 1994; Prince et al., 1992], suggesting that infection with HCV may not induce protective immunity. A recent study of multiple episodes of acute NANB hepatitis in polytransfused thalassemic children indicated that reinfection with different HCV strains can occur in children undergoing long-term transfusion [Lai et al., 1994]. Our previous reports also showed that heterotypic superinfection and mixed infections of HCV may be important in the pathogenesis of hepatitis flares [Kao et al., 1993a,b]. These findings prompted us to investigate the possibility of HCV superinfection, heterotypic or homotypic, in HCV carriers with post-transfusion hepatitis by comparing nucleotide sequences of the hypervariable region (HVR) before and after blood transfusion.

MATERIALS AND METHODS

Patients

Since June 1987, a prospective study of post-transfusion hepatitis was conducted at the National Taiwan University Hospital. The details of patient enrollment

Accepted for publication July 1, 1996.

Address reprint requests to Dr. D.S. Chen, Hepatitis Research Center, National Taiwan University Hospital, 1 Chang-Te St., Taipei, Taiwan 100, Republic of China.

Presented at the 3rd International Meeting on Hepatitis C Virus and Related Viruses, August 30 through September 3, 1995, Gold Coast, Australia.

© 1996 WILEY-LISS, INC.

and interim results have been reported previously [Wang et al., 1992]. Briefly, patients who received blood transfusion and met the following criteria were recruited: normal liver biochemical tests before transfusion; no transfusion in the preceding year; and no previous history of liver disease, alcoholism, drug abuse, or exposure to hepatotoxic drugs. Patients received blood transfusion from volunteer donors negative for hepatitis B surface antigen (HBsAg) and with serum alanine aminotransferase (ALT) level <45 U/liter (normal <31). After transfusion, the recipients were seen every 1 or 2 weeks for the first 3 months and every 2–4 weeks for 4–6 months. Blood samples taken before transfusion and on each visit after transfusion were stored at −80°C until use. Recipients were diagnosed as post-transfusion hepatitis if serum ALT level exceeded 2.5 times the upper normal limit between 1 and 26 weeks after transfusion, at least twice at intervals of 1 or more weeks apart, and if other causes of the elevated serum ALT level were excluded. Hepatitis caused by NANB virus(es) was diagnosed by the absence of immunoglobulin (IgM) antibody to hepatitis B core antigen, IgM antibody to hepatitis A virus, IgM antibody to cytomegalovirus in the acute stage.

As of July 1992 when second-generation antibody to HCV (anti-HCV) was tested in volunteer blood donors, 665 patients, mostly with open heart surgery, completed the 6-month follow-up and 81 of them (12.2%) developed NANB hepatitis. HCV carriage with post-transfusion NANB hepatitis was studied in six patients whose pretransfusion serum samples had already been positive for both anti-HCV and HCV RNA. For these six patients, serum samples from just before transfusion and during hepatitis (2 weeks to 5 months after transfusion) were selected to determine genotypes of HCV as well as nucleotide sequences of the HVR. However, serum samples from the blood donors were not available in this study for comparison of HVR sequences between donors and recipients.

Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Anti-HCV

Serum anti-HCV was tested by a second-generation enzyme immunosorbent assay (Abbott Laboratories, North Chicago, IL) according to the manufacturer's instructions.

Detection of HCV RNA and genotyping of HCV

HCV RNA was examined by reverse transcription–polymerase chain reaction (RT-PCR) with nested primers from the most conserved 5′ non-translating region of the viral genome [Kao et al., 1992], and identification of HCV genotype was by type-specific primers as described previously [Kao et al., 1995b]. The genotype nomenclature was based on a widely accepted system [Simmonds et al., 1994]. Serum samples from patients with defined types 1b, 2a, or 2b virus infection were mixed together and used as positive controls for the PCR assay, while samples from healthy persons and reagents without DNA were used as negative controls. The sensitivity and specificity of this typing assay has been demonstrated [Kao et al., 1994b]. To avoid false-positive results, the method described previously was used to prevent cross contaminations [Kwok and Higuchi, 1989].

Amplification and sequencing of the HVR (nucleotides 1156–1233) [Weiner et al., 1991]

The presence of the N terminus of E2/NS1 region (HVR) was detected by RT-PCR with oligonucleotide primers designed for amplification of the HVR (outer sense: 904 5′-CAGGACTGCAATTGCTCAATCTA-3′; outer antisense: 481 5′-TTGCAGTTTAAGGCAGTCC-3′; inner sense: 1054 5′-CACTGGGGAGTCCTGG-CGGG-3′; inner antisense: 483 5′-ATGTGCCAGCTGC-CATTGGT-3′) [Kao et al., 1993b; 1994a]. The sequences of HVR PCR products were directly determined first by using fluorescence-labeled primers with a 373A Sequencer (Applied Biosystems, Foster City, CA). When needed, cloning and sequencing of the PCR products were performed as previously described [Kao et al., 1995a]. Briefly, the amplified DNA was ligated to pCR-Script™ SK(+) vectors and then transformed to *Escherichia coli* XL-1-Blue MRF′ competent cells (Stratagene, La Jolla, CA). Plasmid DNAs were extracted from white colonies by the Winard™ minipress DNA purification system (Promega, Madison, WI). The presence of insert DNA with anticipated size was confirmed by SacI and BamHI digestion and electrophoresis. Sequencing conditions were specified in the protocol for the Taq Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The primer pair 1054 and 483 was used as sequencing primers for both directions of the HVR.

Phylogenetic Tree Analysis

A phylogenetic tree was constructed by using the maximum-likelihood method (DNAML) available in the PHYLIP package of Felsenstein (version 3.5c, March 1993), based on the nucleotide sequences of the amplified HVR region.

RESULTS

All six patients were negative for serum HBsAg and positive for serum HCV RNA before and after blood transfusion. Their serum ALT levels were all below 31 U/liter before transfusion. The laboratory data of these HCV carriers with post-transfusion NANB hepatitis are shown in Table I. They had a mean peak serum ALT level of 173 U/liter. The HCV genotypes determined by the PCR typing assay before and after transfusion were concordant in all of them with type 1b virus in four, type 2a virus in one, and type 2b virus in one.

The HVR of HCV genome both before transfusion and during hepatitis could be amplified in four (patients 1–4, Table I) and was sequenced directly to determine the master sequence. The comparison of nucleotide sequences between each HCV isolate before transfusion

TABLE I. Laboratory Data of Six Hepatitis C Virus Carriers With Post-Transfusion Hepatitis in a Prospective Study

Patient	Sex	Age (years)	Peak ALT (U/liter)	Genotype	
				Pre	Post
1	M	29	196	1b	1b
2	M	58	126	1b	1b
3	M	57	134	1b	1b
4	M	34	131	2a	2a
5	F	47	334	2b	2b
6	F	36	114	1b	1b

and during hepatitis, and those of the prototype viruses (the Taiwanese HCV genome, a type 1b virus, and the HCV J6 genome, a type 2a virus) is shown in Figure 1. In three of them (patients 1, 2, and 4, Table I), sequence analysis of the HVR (78 nucleotides) showed 4–5% nucleotide variation and 8–15% amino acid variation between the HCV strains isolated with the intervals ranging from 2 weeks to 5 months. In contrast, a high degree (49%) of genetic heterogeneity with amino acid variation of 69% was observed in patient 3 (Table I) between the HCV strains isolated within a period of 2 months.

Although direct sequence analysis can identify the predominant sequence and is useful for detecting variations in the consensus sequence of a population of closely related genomes (quasispecies), the possibility that the post-transfusional isolate (3′, Fig. 1) had already existed as a minor clone of quasispecies before transfusion in patient 3 cannot be excluded. Thus we sequenced 12 recombinant clones obtained from the pretransfusion sample of this patient, and the post-transfusion strain was not found among these clones. To validate further these findings, the evolutionary distance between HCV strains before and after blood transfusion was investigated by phylogenetic analysis of sequences amplified from the HVR region of the HCV genome (Fig. 2). All but patient 3 had similar viral strains before transfusion and during hepatitis, suggesting that patients 1, 2, and 4 had persistence of original viruses while patient 3 was superinfected by a homotypic but evolutionarily distant viruses.

DISCUSSION

Although several patterns of serum ALT activities in chronic hepatitis C have been identified [Alter et al., 1992], the viral mechanisms of fluctuating hepatitis activities are far from clear. Recent studies based on chimpanzee experiments and human observations have shed light on the pathogenesis responsible for episodes of hepatitis flares in chronic HCV carriers [Farci et al., 1992; Kao et al., 1993a, 1994b; Lai et al., 1994; Okamoto et al., 1994; Prince et al., 1992]. In chronic hepatitis B, superinfection with other hepatitis viruses and reactivation of the original hepatitis B virus are two major causes of clinical exacerbations [Chen et al., 1986, 1988; Davis et al., 1984; Lai et al., 1988; Lok and Lai, 1990; Sheen et al., 1992]. Similar mechanisms may also contribute to the acute exacerbations of chronic hepatitis C. Several cross-challenge studies in chimpanzees have provided evidence that reinfection with either homotypic or heterotypic HCV along with chronic HCV carriage does occur [Farci et al., 1992; Okomoto et al., 1994; Prince et al., 1992]. In humans, multiple episodes of acute hepatitis in polytransfused thalassemic children have been reported to be due to reinfection with a different HCV strain in two children, and in the third may be due to reactivation of the primary infection [Lai et al., 1994]. We have demonstrated that superinfection of heterotypic HCV indeed occurs in humans, and mixed infections of heterotypic HCV may be important in causing hepatitis flares in chronic hepatitis C [Kao et al., 1993a, 1994b].

In the present study, we explored further the pathogenic mechanisms of hepatitis flares in six HCV carriers with post-transfusion NANB hepatitis. The results initially obtained by using a PCR typing assay showed that HCV genotypes before and after transfusion were concordant in all six patients (Table I), indicating the absence of reinfection with heterotypic HCV in these patients. On the other hand, the possibility of homotypic HCV superinfection could not be excluded. Although PCR typing methods are useful for classification of HCV genotypes [Chayama et al., 1993; Kao et al., 1995b; McOmish et al., 1993], they fail to differentiate viral strains of the same genotype; for this, analysis of the hypervariable region remains the method of choice [Houghton et al., 1991; Kao et al., 1993a, 1994b]. Thus, we directly sequenced the HVR of HCV genome in four patients with available amplified products, and in three of them the comparative sequence analysis showed a >95% homology between HCV strains isolated before transfusion and during hepatitis. Further comparison of amino acid sequences before transfusion and during hepatitis in these three patients showed the rate of amino acid variation in the HVR domain ranging from 8% to 15% (data not shown), comparable to our recent report that the sequential amino acid variation of the HVR was 15.4% in one chronic hepatitis C patient with acute exacerbation and 2.3% in another without acute exacerbation [Kao et al., 1995a]. These findings strongly suggested that their post-transfusion hepatitis might be caused by the original virus or agents other than HCV. In contrast, although the HVR sequences in the blood donor was not available, both the striking sequence heterogeneity (49% in nucleotide and 69% in amino acid sequences) between the HCV strains isolated in a short interval (2 months) and the absence of post-transfusion strain in pretransfusion clones from a given patient suggested that the hepatitis flare might be induced by reinfection of homotypic HCV.

Immune selection pressure has been implicated as the major driving force for genetic heterogeneity of HCV [Higashi et al., 1993; Kao et al., 1993a, 1995a; Kurosaki et al., 1994; Weiner et al., 1991], but it is hardly conceivable that such a significant genetic variation during a short period of time is simply a result of continuous

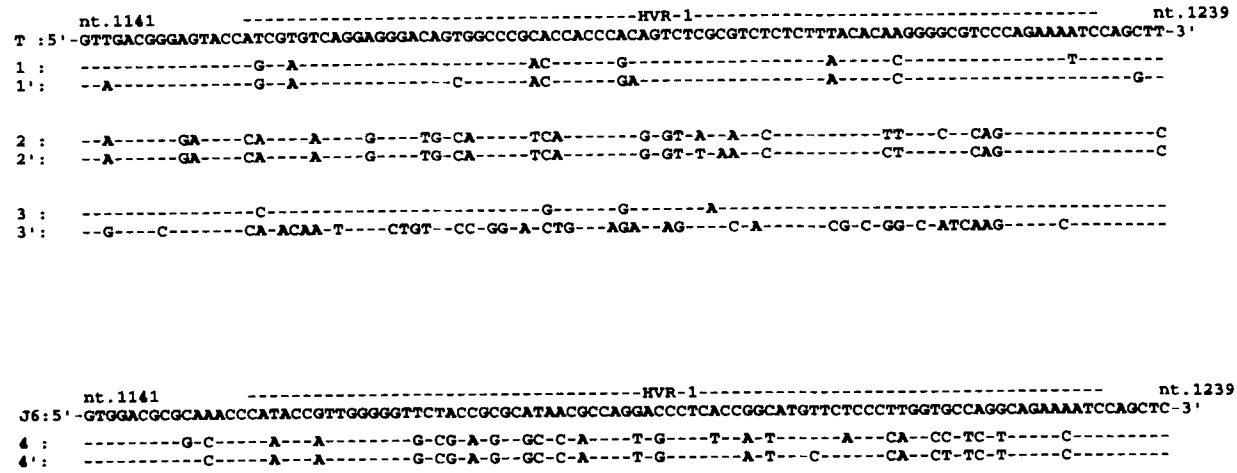

Fig. 1. Comparison of nucleotide sequences of the hypervariable region (HVR) between each HCV strain amplified before transfusion (1–4) and during episodes of hepatitis (1′–4′) from four HCV carriers with post-transfusion NANB hepatitis (patients 1–4, Table I). The Taiwanese genome (T) and Japanese genome (J6) are used as the prototype sequences (type 1b and type 2a, respectively) for comparison. The dotted lines indicate identical sequence compared with the prototypes. Viral strains from patients 1–3 are closer to the Taiwanese genome (type 1b), and that from patient 4 is similar to the Japanese genome (type 2a).

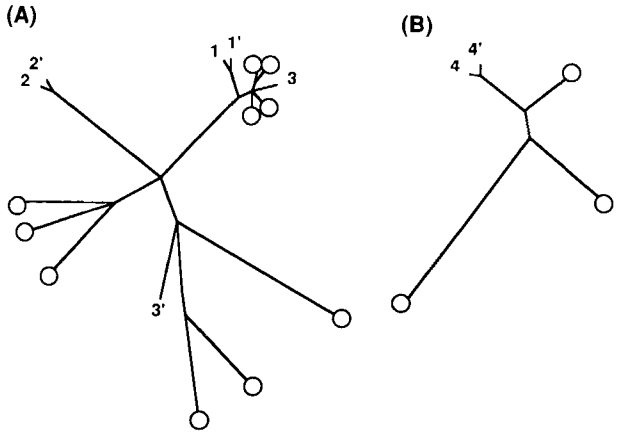

Fig. 2. Phylogenetic trees of HCV type 1b (A) and 2a (B) constructed by the maximum-likelihood method analyzing nucleotide sequences of the HVR region. HCV strains amplified before transfusion (1–4) and during hepatitis (1′–4′) were obtained from four HCV carriers with post-transfusion NANB hepatitis (patients 1–4, Table I). Viral strains from patients 1–3 were type 1b viruses, and that from patient 4 was a type 2a virus. The open circles in (A) and (B) represent non-implicated HCV type 1b and 2a isolates cloned from our laboratory. These trees are not rooted.

accumulation of mutations or selective overgrowth of preexisting minor variants from the large spectrum of quasispecies populations. Furthermore, the phylogenetic tree analysis demonstrated that patients 1, 2, and 4 had similar viral strains before and after transfusion, while the homotypic virus during the hepatitis flare of patient 3 after blood transfusion was evolutionarily distant from that before transfusion. Thus, taking our previous and present data together, these lines of evidence imply that superinfection of HCV, either heterotypic or homotypic, occurs in humans with chronic HCV infection, as was observed in chimpanzee studies [Farci et al., 1992; Okamoto et al., 1994; Prince et al., 1992].

Depressed replication between different hepatitis viruses has been documented [Chen et al., 1988; Davis et al., 1984; Sheen et al., 1992]. As to HCV, previous studies on humans and chimpanzees have demonstrated that when superinfection with heterotypic HCV occurs the new virus may replace or be expelled by the original virus [Farci et al., 1992; Kao et al., 1993a, 1994b; Okamoto et al., 1994], indicating that depressed replication can occur among different genotypes of HCV. Whether such depression exists among homotypic HCV strains remains to be seen. In this study, we found that the newly introduced HCV became the dominant sequence during a hepatitis flare, suggesting that depressed replication among HCV strains of the same genotype can occur. Although the mechanisms of this replacement remain unclear, depressed replication between different HCV strains or host immune response against the original virus should be taken into consideration.

The possibility of surgery-related HCV reactivation must also be addressed. In the Transfusion-Transmitted Viruses Study [Mosley et al., 1991], there were nine patients with NANB hepatitis who were already anti-HCV-positive at entry; five were from 132 blood recipients, and four were from 35 control subjects receiving hospital procedures only (usually a surgical operation) alone. Although the possibility of reinfection with HCV in these five HCV carriers with post-transfusion hepatitis was not addressed in that study, the etiology of hepa-

titis in the four HCV-infected controls who did not receive blood transfusion is of particular interest. Surgery may be important in contributing to the hepatitis which is caused by HCV reactivation as observed in our three patients (patients 1, 2, and 4). Further characterization of the hepatitis in untransfused hospitalized HCV carriers is needed to clarify this interesting and important issue.

In summary, our data imply that chronic HCV carriers can be superinfected by heterotypic HCV as well as by homotypic HCV, and this may contribute to hepatitis flares in patients with chronic hepatitis C. These findings further confirm a weak or inadequate protective immunity in HCV infection and justify protection from reinfection with HCV in patients with chronic hepatitis C. These observations also cast doubt on the possibility of effective vaccination against HCV infection.

ACKNOWLEDGMENTS

This study was supported by grants from the Department of Health and the National Science Council, Executive Yuan, ROC.

REFERENCES

Alter H (1990): The hepatitis C virus and its relationship to the clinical spectrum of NANB hepatitis. Journal of Gastroenterology and Hepatology 5(Suppl 1):78–94.

Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE, Meeks EL, Beach MJ (1992): The natural history of community-acquired hepatitis C in the United States. New England Journal of Medicine 327:1899–1905.

Bukh J, Miller RH, Purcell RH (1995): Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. Seminars in Liver Disease 15:41–63.

Chayama K, Tsubota A, Arase Y, Saitoh S, Koida I, Ikeda K, Matsumoto T, Kobayashi M, Iwasaki S, Koyama S, Morinaga T, Kumada H (1993): Genotypic subtyping of hepatitis C virus. Journal of Gastroenterology and Hepatology 8:150–156.

Chen DS (1994): Hepatitis B and C virus infections in hepatocellular carcinoma and their prevention. In Nishioka K, Suzuki H, Mishiro S, Oda T, (eds): "Viral Hepatitis and Liver Disease." Tokyo: Springer-Verlag, pp 685–689.

Chen DS, Lai MY, Lee SC, Yang PM, Sheu JC, Sung JL (1986): Serum HBsAg, HBeAg, anti-HBe, and hepatitis B viral DNA in asymptomatic carriers in Taiwan. Journal of Medical Virology 19:87–94.

Chen PJ, Chen DS, Chen CR, Chen YY, Chen Hsu HM, Lai MY, Sung JL (1988): δ infection in asymptomatic carriers of hepatitis B surface antigen: low prevalence of δ activity and effective suppression of hepatitis B virus replication. Hepatology 8:1121–1124.

Chen PJ, Lin MH, Tai KF, Liu PC, Lin CJ, Chen DS (1992): The Taiwanese hepatitis C virus genome: sequence determination and mapping the 5′ termini of viral genomic and antigenomic RNA. Virology 188:102–113.

Davis GL, Hoofnagle JH, Waggoner JG (1984): Acute type A hepatitis during chronic hepatitis B virus infection: association of depressed hepatitis B virus replication with appearance of endogenous alpha interferon. Journal of Medical Virology 14:141–147.

Farci P, Alter HJ, Govindarajan W, Wong DC, Engle R, Lesniewski RR, Mushahwar IK, Desai SM, Miller RH, Ogata N, Purcell RH (1992): Lack of protective immunity against reinfection with hepatitis C virus. Science 258:135–140.

Higashi Y, Kakumu S, Yoshioka K, Wakita T, Mizokami M, Ohba K, Ishikawa YT, Takayanagi M, Nagai Y (1993): Dynamics of genome change in the E2/NS1 region of hepatitis C virus in vivo. Virology 197:659–668.

Houghton M, Weiner A, Han J, Kuo G, Choo QL (1991): Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology 14:381–388.

Kao JH, Chen PJ, Yang PM, Lai MY, Sheu JC, Wang TH, Chen DS (1992): Intrafamilial transmission of hepatitis C virus: the important role of infections between spouses. Journal of Infectious Diseases 166:900–903.

Kao JH, Chen PJ, Lai MY, Chen DS (1993a): Superinfection of heterologous hepatitis C virus in a patient with chronic type C hepatitis. Gastroenterology 105:583–587.

Kao JH, Chen PJ, Lai MY, Wang TH, Chen DS (1993b): Sexual transmission of HCV. Lancet 342:626.

Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH, Chen DS (1994a): Detection of divergent hepatitis C virus envelope sequences. Journal of Biomedical Science 1:158–162.

Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH, Chen DS (1994b): Mixed infections of hepatitis C virus as a factor in acute exacerbation of chronic type C hepatitis. Journal of Infectious Diseases 170:1128–1133.

Kao JH, Tsai SL, Chen PJ, Yang PM, Sheu JC, Lai MY, Hsu HC, Sung JL, Wang TH, Chen DS (1994c): A clinicopathologic study of chronic non-A, non-B (type C) hepatitis in Taiwan: comparison between posttransfusion and sporadic patients. Journal of Hepatology 21:244–249.

Kao JH, Chen PJ, Lai MY, Wang TH, Chen DS (1995a): Quasispecies of hepatitis C virus and genetic drift of the hypervariable region in chronic type C hepatitis. Journal of Infectious Diseases 172: 261–264.

Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH, Chen DS (1995b): Genotypes of hepatitis C virus in Taiwan and the progression of liver disease. Journal of Clinical Gastroenterology. 21:233–237.

Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ (1990): Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 12:671–675.

Kurosaki M, Enomoto N, Marumo F, Sato C (1994): Evolution and selection of hepatitis C virus variants in patients with chronic hepatitis C. Virology 205:161–169.

Kwok S, Higuchi R (1989): Avoiding false positive with PCR. Nature 339:237–238.

Lai MY, Chen DS, Lee SC, Su IJ, Yang PM, Hsu HC, Sung JL (1988): Reactivation of hepatitis B virus in anti-HBe-positive chronic active type B hepatitis: molecular and immunohistochemical studies. Hepatogastroenterology 35:17–21.

Lai ME, Mazzoleni AP, Argiolu F, De Virgilis S, Balestrieri A, Purcell RH, Cao A, Farci P (1994): Hepatitis C virus in multiple episodes of acute hepatitis in polytransfused thalassaemic children. Lancet 343:388–390.

Lok ASF, Lai CL (1990): Acute exacerbations in Chinese patients with chronic hepatitis B virus (HBV) infection. Incidence, predisposing factors and etiology. Journal of Hepatology 10:29–34.

Martell M, Esteban JI, Quer J, Genesca J, Weiner A, Esteban R, Guardia J, Gomez J (1992): Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. Journal of Virology 66: 3225–3229.

McOmish F, Chan SW, Dow BC, Gillon J, Frame WD, Crawford RJ, Yap PL, Follett EAC, Simmonds P (1993): Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serologic reactivity and rate of alanine aminotransferase abnormalities. Transfusion 33:7–13.

Mosley JW, Stevens CE, Aach RD, Hollinger FB, Barbosa LH (1991): Role of hepatitis C virus infection in transfused-associated non-A, non-B hepatitis. In Hollinger FB, Lemon SM, Margolis HS (eds): "Viral Hepatitis and Liver Disease." Baltimore: Williams and Wilkins, pp 402–407.

Okamoto H, Okada S, Sugiyama Y, Kurai K, Iizuka H, Machida A, Miyakawa Y, Mayumi M (1991): Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: comparison with reported isolates for conserved and divergent regions. Journal of General Virology 72:2697–2704.

Okamoto H, Mishiro S, Tokita H, Tsuda F, Miyakawa Y, Mayumi M (1994); Superinfection of chimpanzees carrying hepatitis C virus of genotype II/1b with that of genotype III/2a or I/1a. Hepatology 20:1131–1136.

Prince AM, Brotman B, Huima T, Pascual D, Jaffery M, Inchauspe G (1992): Immunity in hepatitis C infection. Journal of Infectious Diseases 165:438–443.

Sheen IS, Liaw YF, Chu CH, Pao CC (1992); Role of hepatitis C virus

infection in spontaneous hepatitis B surface antigen clearance during chronic hepatitis B virus infection. Journal of Infectious Diseases 165:831–834.

Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS (1993); Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylotenetic analysis of the NS-5 region. Journal of General Virology 74:2391–2399.

Simmonds P, Alberti A, Bonino F, Bradley DW, Brechot C, Chan SW, Chayama K, Chen DS, Choo QL, Colombo M, Cuypers TM, Date T, Esteban JI, Osker F, Hans J, Holmes EC, Hotta H, Houghton M, Irvine B, Kohara M, Kolberg J, Lau JYN, Lelie PN, McOmish F, Miyamura M, Mizokami M, Nomoto A, Prince AM, Reesink HW, Rice C, Roggendorf M, Shimotono K, Stuyver L, Trepo C, Weiner A, Yap PL, Urdea MS (1994): A proposed system for the nomenclature for genotypes of hepatitis C virus. Hepatology 19:1321–1324.

Wang JT, Wang TH, Sheu JC, Lin JT, Wang CY, Chen DS (1992): Posttransfusion hepatitis revisited by hepatitis C antibody assays and polymerase chain reaction. Gastroenterology 103:609–616.

Weiner AJ, Braner M, Rosenblatt J, Richman K, Tung J, Crawford K, Bonino F, Saracco G, Choo QL, Houghton M, Han JH (1991): Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS 1 proteins and the pestivirus envelope glycoproteins. Virology 180:842–848.